

THE CURRENT STATUS OF *CYPRIPEDIUM*
KENTUCKIENSE (ORCHIDACEAE)
INCLUDING A MORPHOLOGICAL ANALYSIS
OF A NEWLY DISCOVERED POPULATION
IN EASTERN VIRGINIA

TROY W. WELDY, HENRY T. MLODOZENIEC,
LISA E. WALLACE, and MARTHA A. CASE¹

Department of Biology
The College of William & Mary
Williamsburg, VA 23187-8795, U.S.A.

ABSTRACT

In 1995, a population of 120 very large yellow lady's slippers was discovered in a remote ravine on the Northern Neck peninsula of Eastern Virginia. Multivariate and univariate analyses of morphological variation in the Virginia population suggest that these individuals belong to a rare species, *Cypripedium kentuckiense*, and not the more common yellow lady's slipper, *C. parviflorum* var. *pubescens*. These analyses also indicate that the dorsal sepal width and orifice length are two important characters that can be used to discriminate between *C. kentuckiense* and *C. parviflorum* var. *pubescens*. A few individuals at the Virginia site approached the size of *C. parviflorum* var. *pubescens* in some characters which may be due to historical introgressive patterns or genetic isolation. Lastly, a survey of records on *C. kentuckiense* populations revealed that Arkansas contains the largest number of populations (70 out of 156). These records also indicate that *C. kentuckiense* is characterized by a majority of populations (58%) with less than 21 individuals.

RESUMEN

En 1995 se descubrió una población de 120 individuos de zapato de venus ("yellow lady's slippers" en inglés) en un barranco alejado localizado en la península llamada "Northern Neck" del este del estado de Virginia, EEUU. Un análisis multivariante y univariante de la variación morfológica en la población investigada de Virginia sugiere que estos especímenes pertenecen a una especie rara, *Cypripedium kentuckiense*, en vez de la especie más común de zapato de venus *C. parviflorum* var. *pubescens*. Este análisis también indica que la anchura del sépalo dorsal y la longitud del orificio son dos características importantes que pueden ser usadas para distinguir *C. kentuckiense* de *C. parviflorum* var. *pubescens*. Unos especímenes del lugar de investigación en Virginia se aproximan al tamaño de *C. parviflorum* var. *pubescens* en algunas características que pueden explicarse debido a patrones históricos introgresivos o aislamiento genético. Por último, una revisión del conjunto de investigaciones sobre poblaciones de *C. kentuckiense* reveló que el estado de Arkansas contiene el número más grande de poblaciones (70 de 156). Estas investigaciones también indican que *C. kentuckiense* está caracterizado por una mayoría de poblaciones (58%) con menos de 21 especímenes.

¹Corresponding Author

INTRODUCTION

The *Cypripedium calceolus* L. complex in North America is currently thought to comprise four species and three varieties (Sheviak 1992, 1994). It includes the yellow lady's slipper taxa [*C. parviflorum* var. *parviflorum* Salisb., *C. parviflorum* var. *pubescens* (Willd.) Knight, and *C. parviflorum* var. *makasin* (Farw.) Sheviak] as well as lady's slipper taxa that contain white and creamy white labella (*C. montanum* Douglas ex Lindley, *C. candidum* Muhlenb. ex Willd., and *C. kentuckiense* C. Reed). *Cypripedium parviflorum* occupies the largest range of all species in the North American group, occurring in approximately 40 states and throughout most of Canada (Luer 1975). In addition to its large range, *C. parviflorum* displays high levels of morphological variation which has led to the delimitation of many specific and subspecific taxa (see review in Newhouse 1976). The delimitation of taxa within *C. parviflorum* is complicated by complex hybridization patterns among its varieties and related species (e.g., Klier et al. 1991; Case 1993), regional variation in reproductive isolating mechanisms (Case 1993), and very high levels of genetic variation (Case 1994). The extensive morphological variation and complex breeding patterns of *C. parviflorum* and related taxa have caused them to be the subject of many taxonomic and evolutionary debates that have persisted for more than 200 years.

One of the most recently described species in the N. American group is the relatively rare *C. kentuckiense*. This species most closely resembles *C. parviflorum* var. *pubescens* in morphology and appears to be a taxon that was recently derived from *C. parviflorum* var. *pubescens* (Case 1994). Although *C. kentuckiense* was not validly described until 1981, its morphological concept may date back to an entity described by Rafinesque in 1828, named *C. luteum* var. *grandiflorum* Raf. However, the lack of a type specimen designation for the Rafinesque name precludes a definitive association between this name and a biological entity (Atwood 1984). Prior to its valid description by Reed, *C. kentuckiense* was considered part of one polymorphic North American lady's slipper species (e.g., Correll 1950) or considered to be a distinct species [Soukup (1977) invalidly described this species under the name *C. daultonii*].

Shortly after its valid description, Atwood (1985) conducted a study to clarify the range of *C. kentuckiense* by examining herbarium and live specimens from the southeastern United States. He concluded that Arkansas had the largest number of localities (11), but suggested that this might be an artifact due to less collection effort in some states [e.g., Mississippi (1) and Alabama (1)]. He also found locations for *C. kentuckiense* in Oklahoma (4), Texas (2), Louisiana (5), Tennessee (1), and Kentucky (4), but did not find this taxon represented in the Carolinas or Virginia. He suggested that

conservationists be on the lookout for this taxon in Missouri, Illinois, Indiana, Ohio, West Virginia, and Georgia. Since then, there have been numerous attempts to find *C. kentuckiense* in these bordering states, but there are no documented records of any discoveries to date (Larry Morse pers. comm.). Currently, the global rank of *C. kentuckiense* indicates that it is threatened throughout its range (G3), while the federal ranking (C2) indicates that it is a likely candidate for the list of federal endangered and threatened species. However, its C2 rank also implies that it will be necessary to obtain further biological information pertaining to potential threats to the species to determine whether or not it should be placed on the federal list (Department of the Interior 1993). At the state level, *C. kentuckiense* is most often given S1 status (i.e., critically imperiled, extremely rare, and very vulnerable to extirpation; Table 1).

In 1995, a population of *C. kentuckiense* was discovered (Weldy 1995) on the Northern Neck peninsula of eastern Virginia (between the Rappahannock and Potomac rivers), approximately 285 mi from the nearest known *C. kentuckiense* locality in northeastern Kentucky. The population contains approximately 120 individuals and is located along a sandy ravine bottom adjacent to a small stream (hereafter the population is called the Virginia population). The most notable aspects of the Virginia population are the large overall size of the individuals and their creamy yellow labella (Fig. 1). The latter characteristic is far more typical of *C. kentuckiense* than *C. parviflorum* var. *pubescens*, which usually has bright yellow labella. Although this population strongly resembles *C. kentuckiense*, some individuals approach the size of *C. parviflorum* var. *pubescens*, which is a smaller taxon. Therefore, the first objective of this paper is to quantify the morphological variation within the Virginia population of *C. kentuckiense* in relation to other *C. kentuckiense* and *C. parviflorum* var. *pubescens* populations. Our second objective is to review the number of populations, population sizes, and locations of all *C. kentuckiense* populations. This review is intended to provide a phytogeographical perspective for the Virginia population and provide an updated review of the national status of *C. kentuckiense*.

MATERIALS AND METHODS

To obtain information on the current status of *Cypripedium kentuckiense* populations, element occurrence records were examined from Natural Heritage offices in states with known localities. These states included Alabama, Arkansas, Kentucky, Louisiana, Mississippi, Oklahoma, Tennessee, and Texas. In addition, Heritage offices in states adjacent to those containing *C. kentuckiense* populations were contacted for data on *C. kentuckiense* sites. These included Georgia, Illinois, Indiana, Missouri, North Carolina, Ohio, South Carolina, Virginia, and West Virginia. For each state, records were



FIG. 1. *Cypripedium kentuckiense* in Virginia. Photograph by T. Weldy.

obtained on the number of known populations and the number of individuals in each population. When state records included a range for any given population size, the maximum population size was used in our review. If information from more than one survey was indicated for any given population, the most recent record that reported the number of individuals in the population was used. It should be noted, however, that in some cases the most recent record was an original record that was unverified by Heritage botanists. Therefore, the number of populations reported in this study may be an overestimate due to the inclusion of records that have not recently been verified. Populations for which no data were given on the number of individuals present were included in the total count of populations but were not included in any other statistics. Thirty-five percent of all populations reported did not contain census data on the number of individuals present. Therefore, the numbers of individuals per state given in Table 1 are likely to be underestimates of the actual number per state.

To establish the taxonomic identity of the Virginia population, multivariate and univariate analyses of 13 morphological characters were conducted. Nine of these characters were used by Reed (1981) in his diagnosis of *C. kentuckiense*. The specimens used in this analysis included 14 individuals from the Virginia population of *C. kentuckiense*, 5 individuals from 5 *C. kentuckiense* populations in Texas and Arkansas, and 35 individuals from 13 populations of *C. parviflorum* var. *pubescens* from Michigan, Missouri, Georgia, Indiana, and Virginia. For each specimen, the following morphological characters were measured: plant height (to the top of the ovary), staminode length, length and width of the longest leaf, length and width of the petals, length and width of the dorsal sepal, length and width of the labellum, length and width of the labellum orifice, and the number of 180° turns in the lateral petals. Those characters in this analysis that were not used by Reed (1981) include the orifice dimensions, staminode length, and the number of 180° turns in the lateral petals. In addition, we noted labellum color in the field but did not include it in the quantitative analyses. All measurements were made on living specimens in natural populations by L. E. Wallace, M. A. Case, or H. T. Mlodozieniec.

Multivariate analysis of morphological characters consisted of a principal components analysis (PCA) using NTSYS (Rohlf 1988) to evaluate the existence of natural groupings among all 54 specimens. A variance/covariance matrix was calculated from standardized measurements (i.e., these measurements were in units of standard deviation from the mean) and the first two principal axes were extracted. The axis coordinates for all the individuals were subsequently plotted. For univariate statistical analyses, the existence of unequal sample variances, non-normal sample distributions, and a relatively small sample size of the western *C. kentuckiense* group prohibited the use of parametric tests. Therefore, a Kruskal-Wallis non-parametric mean rank test (Sokal & Rohlf 1995) was applied to test for character differences among the three groups. These groups were defined as the Virginia population, the western *C. kentuckiense* individuals, and all *C. parviflorum* var. *pubescens* individuals. Subsequently, a Dunn's non-parametric multiple comparisons test was used to test all pairwise comparisons among the groups (Zar 1996). The latter was applied to those characters that displayed overall significant differences among groups in the Kruskal-Wallis test. Arithmetic means, standard errors, and ranges of the characters were also calculated using EXCEL (Microsoft 1993).

RESULTS

The survey of element occurrence records revealed that there are 156 populations of *Cypripedium kentuckiense* known. Arkansas had the largest number of populations as well as 66.4% of all reported individuals (Table 1). Ken-

tucky had the second largest number of populations (36) and Louisiana had the third largest number (19). However, the mean population size in Louisiana (6) is very small compared to the mean population sizes reported in other states (17–117). Therefore, Louisiana contains only 1.5% of all reported individuals. Only 9 populations of *C. kentuckiense* are known from Tennessee, but this state contains 13.4% of all reported individuals. This is due to the relatively large population sizes found in Tennessee. Lastly, the majority (58%) of population sizes reported from all states ranged between 1–20 individuals (Fig. 2). Furthermore, 43% of all populations had only 1–10 individuals.

The PCA revealed two distinct groupings on axis 1 which separates *C. parviflorum* var. *pubescens* from *C. kentuckiense* individuals (Fig. 3). The first axis explained 72% of the variation whereas the first two axes combined explained 81% of the variation. With the exception of the degree of petal turns which had a low negative correlation with the first axis (-0.11), all remaining characters had strong positive correlations with the first axis. These values ranged from 0.70 for leaf length to 0.96 for dorsal sepal width. The remaining characters all had correlation values greater than 0.81.

Results of the univariate statistical tests parallel the multivariate results. With the exception of the degree of petal turns which was not significantly different among groups ($p > 0.05$), highly significant differences ($p < 0.001$; Kruskal-Wallis test) were found among groups for all other characters. In pairwise group comparisons for these twelve significant characters (Dunn's non-parametric multiple comparisons test), *C. kentuckiense* from Arkansas and Texas were never significantly different from *C. kentuckiense* from Virginia. In contrast, each of these two *C. kentuckiense* groups were highly significantly different from the *C. parviflorum* var. *pubescens* group (Table 2). In general, the upward range limits of character measurements for *C. parviflorum* var. *pubescens* overlap slightly with the lower range limits of characters recorded for the two *C. kentuckiense* groups. For a few characters (e.g., dorsal sepal width and orifice length) the measurement ranges of *C. parviflorum* var. *pubescens* relative to the *C. kentuckiense* groups were well separated and non-overlapping. These multivariate and univariate statistical results reflect the large size of most *C. kentuckiense* individuals relative to *C. parviflorum* var. *pubescens* individuals.

DISCUSSION

The morphological evidence presented in this paper indicates that the Virginia population is best interpreted as a disjunct population of *C. kentuckiense*, and not as a new taxon or population of large *C. parviflorum* var. *pubescens*. Our multivariate and univariate data analyses are consistent with the hypothesis of two morphological groups. In all group comparisons where

TABLE 1. Distribution and density statistics for *Cypripedium kentuckiense* populations throughout its known range. The information provided includes: the state where populations are located (first column); the state occurrence rank for *C. kentuckiense* [second column (S1 = critically imperiled in state, extremely rare, and very vulnerable to extirpation; SU = possibly in peril in state, but status uncertain; S3 = rare or uncommon in state)]; the month and year the data were obtained (third column); the total number of known populations per state and percent of all populations found within each state (fourth column); the total number of reported individuals per state and percent of all individuals found within each state (fifth column); and the mean±standard deviation, and range of population sizes calculated from occurrence records (sixth column). Sixty five percent of all occurrence records included census data on the number of individuals per population. Summary statistics are in the totals row.

State	Rank	Date	# Pops. & (% of Total)	# Individuals & (% of Total)	Mean±sd & (Range of Pop. Sizes)
AK	S3	4/95	70 (44.9)	2,884 (66.4)	60±94 (1–450)
KY	S3	10/95	36 (23.1)	380 (8.8)	17±22 (1–100)
LA	S1	10/95	19 (12.2)	67 (1.5)	6±5 (1–15)
TN	S1	10/95	9 (5.7)	584 (13.4)	117±108 (14–300)
TX	S1	3/95	8 (5.1)	110 (2.5)	18±15 (3–42)
OK	S1	3/95	7 (4.5)	148 (3.4)	21±25 (3–60)
AL	S1	10/95	4 (2.6)	—	—
MS	SU	3/95	2 (1.3)	50 (1.2)	—
VA	—	—	1 (0.6)	120 (2.8)	—
Totals or Mean	—	—	156 (100)	4,343 (100)	42±75 (1–450)

overall significant differences exist, no significant differences were found between the *C. kentuckiense* and Virginia population comparisons whereas *C. kentuckiense* and the Virginia population were always significantly different from *C. parviflorum* var. *pubescens* (Table 2). Furthermore, the dimensions of characters measured for each taxon are generally within the ranges previously reported (Table 2). However, the dimensions of eight characters measured for *C. kentuckiense* individuals fell outside the ranges previously published for this taxon in Reed (1981) or Gleason and Cronquist (1991). These characters and their corresponding maximum or minimum values were height (53 cm; Virginia population), leaf width (14 cm; Virginia population), petal length (14 cm; Virginia population), petal width (1.0 cm; Virginia and western *C. kentuckiense*), dorsal sepal length (10.5 cm; western *C. kentuckiense*), dorsal sepal width (5 cm; Virginia population), labellum length (6.3 cm; western *C. kentuckiense*), and labellum width (4.5 cm; Virginia population). It is important to note that these values substantially increase the previously published ranges for these characters and that the Virginia population as well as the more western *C. kentuckiense* populations contributed to these increases in character ranges. A substantial increase in

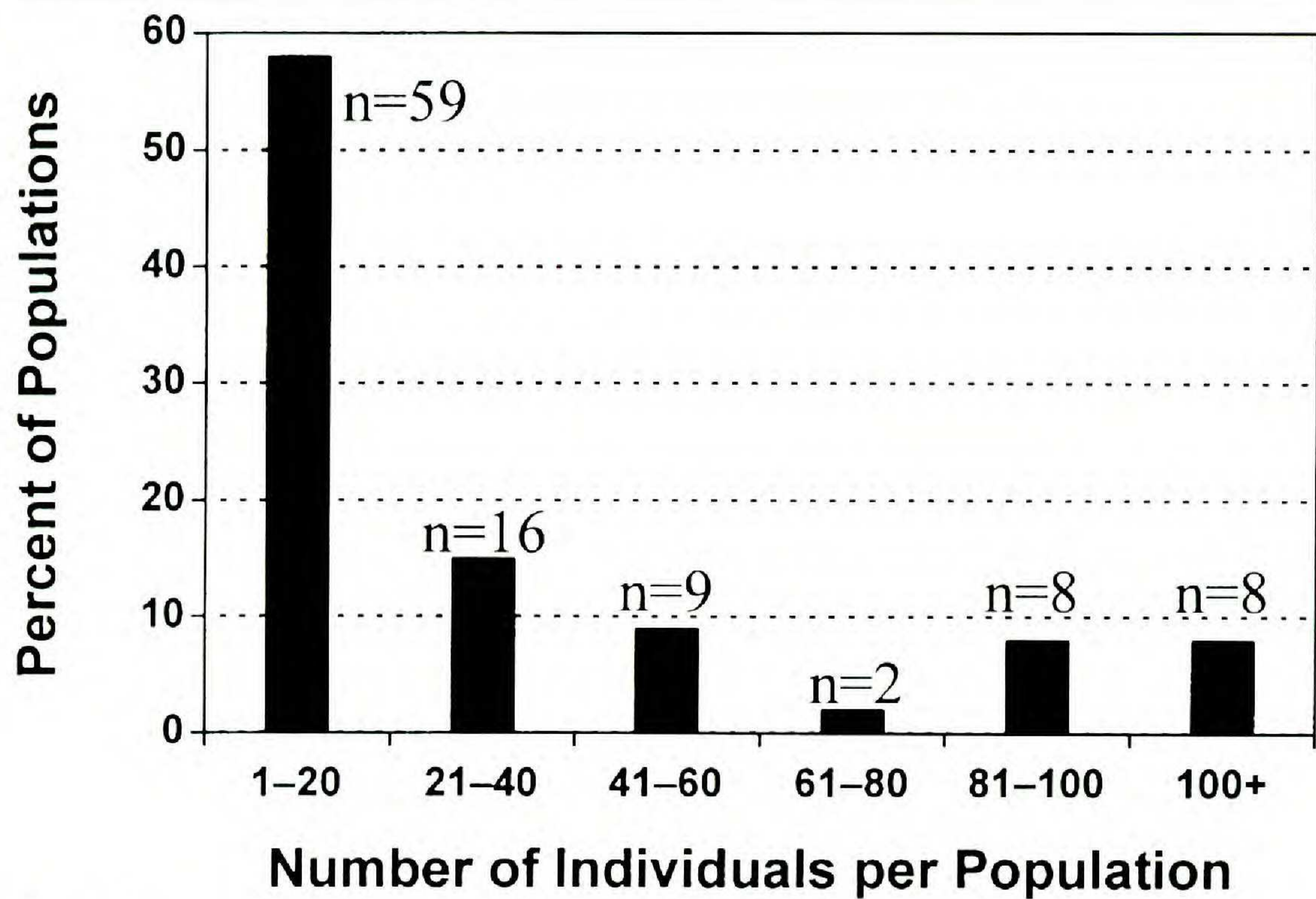


FIG. 2. Distribution of population sizes for 102 (65%) *Cypripedium kentuckiense* population records reporting sizes.

the ranges of characters for *C. kentuckiense* was also found by Sheviak in his treatment of *Cypripedium* for Flora North America (Sheviak pers. comm.; Table 2). The results of the present study and Sheviak's results indicate that prior sampling of *C. kentuckiense* has not been sufficient to determine the morphological ranges of these characters. Therefore, we recommend that floristic researchers be aware of the changing concept of character variation in this taxon. Moreover, our analysis indicates that dorsal sepal width and orifice length may be the most important characters for discriminating between *C. kentuckiense* and *C. parviflorum* var. *pubescens*. These were the only two characters in our study that did not overlap between these two species (Table 2).

Qualitative floral traits of the Virginia population are also consistent with traits found in more western sites. First, the Virginia population is composed of individuals with creamy yellow labella, dark maroon petals and sepals, and dorsal sepals with prominent green and maroon striations near the sepal base (Fig. 1). These characteristics were always present in the five *C. kentuckiense* populations visited in Texas and Arkansas, but they were absent or rare in the *C. parviflorum* var. *pubescens* populations visited (Mlodozieniec pers. obs.). Furthermore, the habitat of the Virginia population (i.e., sandy substrate in the flood plains of creeks) is consistent with

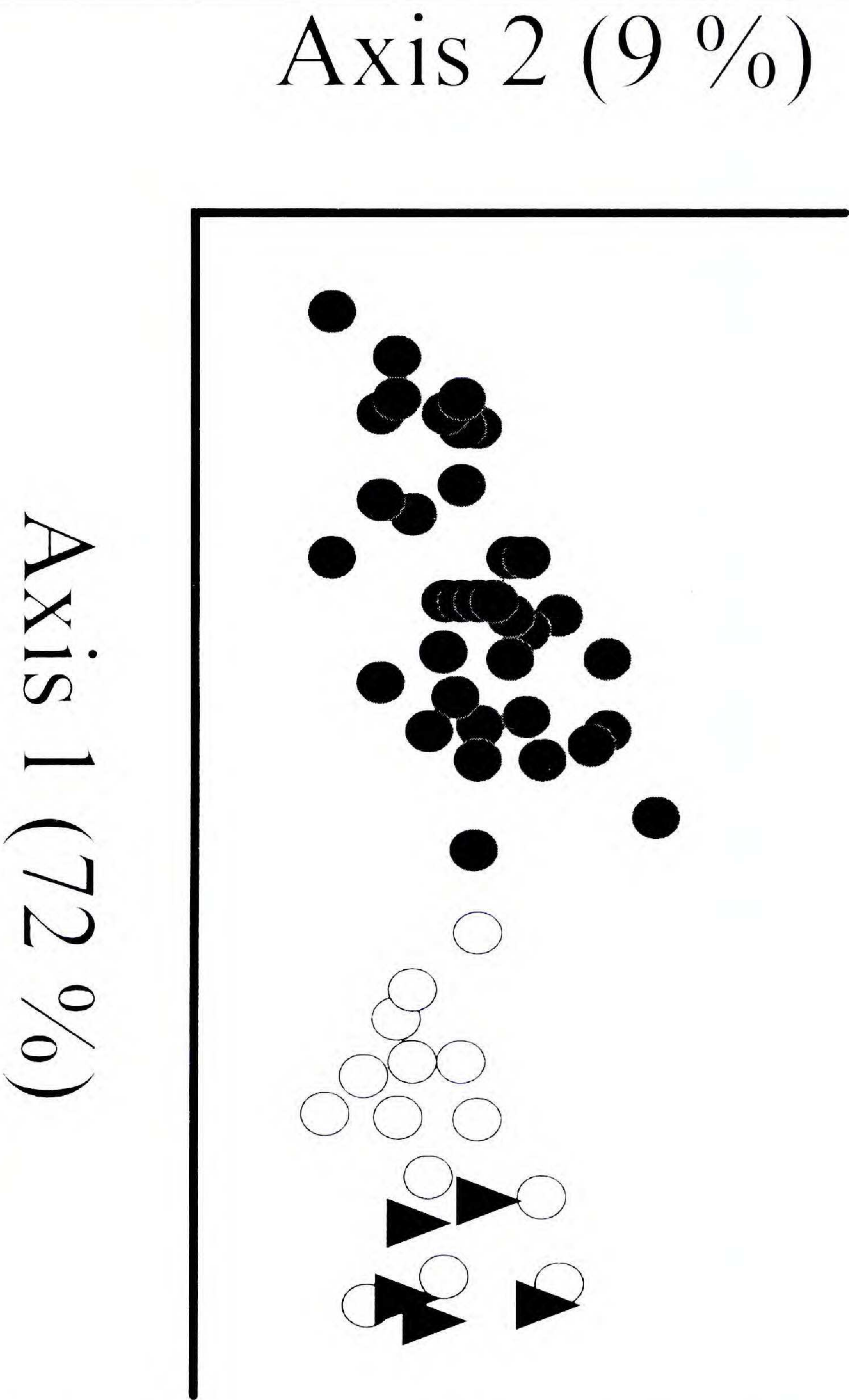


FIG. 3. Principal components analysis of 35 *Cypripedium parviflorum* var. *pubescens* individuals (black circles), 5 *C. kentuckiense* individuals from Arkansas and Texas (black triangles), and 14 *C. kentuckiense* from Virginia (white circles). The amount of variation explained by axis 1 and axis 2 is given in parentheses. The morphological characters used in this analysis are given in Table 2.

TABLE 2. Arithmetic mean±standard errors and ranges (in parentheses) of morphological characters obtained from 54 living specimens, and combined ranges of characters reported by Gleason and Cronquist (1991)¹, Sheviak (pers. comm.)², and/or Reed (1981)³. Measurements are in cm. Statistical groups include *Cypripedium parviflorum* var. *pubescens* from all populations (PUB), *C. kentuckiense* from Texas and Arkansas (KENT), and the Virginia population of *C. kentuckiense* (VA). Different superscript letters among groups within a morphological character indicate significant differences in mean rank scores among pairwise group comparisons at $p < 0.005$ (*) or $p < 0.001$ (**) levels (Dunn's non-parametric multiple comparison test for Kruskal-Wallis mean rank test).

Character & ranges of measurements previously reported	Measurements from this study (individuals measured indicated by n)		
	PUB (n=35)	KENT (n=5)	VA (n=14)
Height PUB (20–80) ¹ ; KENT (35–97) ²	^{a**} 45.7±1.5 (27.8–60.5)	^b 67.0±1.6 (63.0–70.0)	^b 60.8±1.5 (53.0–68.0)
Leaf length PUB (6–20) ¹ ; KENT (13–24) ²	^{a*} 14.6±0.4 (9.0–21.0)	^b 19.5±0.4 (18.0–20.0)	^b 17.6±0.5 (15.0–20.0)
Leaf width KENT; (4.3–15) ²	^{a**} 6.9±0.4 (2.8–10.5)	^b 11.5±0.4 (10.5–13.0)	^b 10.6±0.5 (8.0–14.0)
Staminode length	^{a**} 1.2±0.03 (0.8–1.7)	^b 1.9±0.2 (1.5–2.5)	^b 1.6±0.06 (1.5–2.0)
Petal length PUB (5–8) ¹ ; KENT (8–15.6) ^{1,2}	^{a**} 7.0±0.3 (4.4–10.5)	^b 11.5±0.3 (10.5–12.5)	^b 11.4±0.3 (9.5–14.0)
Petal width KENT (0.7–1.5) ²	^{a*} 0.7±0.03 (0.5–1.0)	^b 1.0±0 —	^b 1.0±0 —
Dorsal sepal length PUB (3–8) ¹ ; KENT (6.1–12.6) ²	^{a**} 5.5±0.2 (3.5–7.5)	^b 9.4±0.3 (9.0–10.5)	^b 8.8±0.2 (7.5–10.0)
Dorsal sepal width KENT (2.4–6.5) ²	^{a**} 2.2±0.06 (1.5–2.9)	^b 4.5±0 —	^b 4.1±0.1 (3.5–5.0)
Labellum length PUB (3–5) ¹ ; KENT (4.1–6.5) ²	^{a**} 3.8±0.1 (2.5–5.8)	^b 5.9±0.2 (5.5–6.3)	^b 5.5±0.1 (4.5–6.0)
Labellum width KENT (3.5) ³	^{a**} 2.2±0.05 (1.5–3.0)	^b 3.9±0.06 (3.7–4.0)	^b 3.5±0.1 (3.0–4.5)
Orifice length KENT (2.7–3.7) ²	^{a**} 0.9±0.03 (0.5–1.3)	^b 3.9±0.06 (3.7–4.0)	^b 3.5±0.1 (3.0–4.5)
Orifice width	^{a**} 1.0±0.04 (0.5–1.5)	^b 2.2±0.2 (1.5–2.5)	^b 1.8±0.08 (1.4–2.5)
Number of 180° turns in petals	^a 3.7±0.2 (1–7)	^a 3.2±0.5 (2–5)	^a 2.7±0.4 (0–5)

the habitat that is typically reported for *C. kentuckiense* in the eastern portion of its range (Whitlow 1986).

Even though no significant differences exist in the morphological characters measured among the Virginia site and the more western sites, the range of character measurements were generally much larger in the Virginia population than in the more western sites (Table 2). This, in part,

could be due to a relatively small sample size of the western *C. kentuckiense*. Alternatively, the Virginia population may be composed of individuals that differ slightly from populations in the remainder of the species' range. A long period of genetic isolation or introgression with *C. parviflorum* var. *pubescens* might have contributed to this pattern. Although non-overlapping groups were formed by the PCA, one Virginia *C. kentuckiense* individual clustered very close to an individual of *C. parviflorum* var. *pubescens* (Fig. 3). This *pubescens* individual was from a population that was 95 mi from the *C. kentuckiense* site. The similar PCA clustering region of these two individuals might indicate historical patterns of introgression. However, Weldy (1995) did not find *C. parviflorum* var. *pubescens* at the Virginia site or in adjacent ravines. In addition, high levels of year-to-year morphological variation within the same individual of *C. kentuckiense* have been observed by Sheviak (pers. comm.). Similar non-genetic contributions to phenotype might also explain why some individuals of *C. kentuckiense* approach the dimensions of *C. parviflorum* var. *pubescens*. Research is currently in progress to address hypotheses concerning the population genetic characteristics of *C. kentuckiense* and *C. parviflorum* var. *pubescens* in Virginia.

It is interesting to note that the Virginia population contains approximately 120 individuals. This large population size is relatively uncommon for this species (Fig. 2) and might be considered especially unusual for a population at the fringe of its species' range. Most documented *C. kentuckiense* populations contain fewer than 21 individuals. Although small population sizes can be caused by many contributing factors (e.g., Harper 1987), we hypothesize that small *C. kentuckiense* population sizes are, in part, due to low rates of population growth. Life history characteristics that could contribute to low rates of population growth include a low rate of seed germination, high seed-to-adult mortality, and a long development time from seed to flowering adult. Furthermore, high seed dispersal rates are expected in *C. kentuckiense* due to its highly reduced wind-dispersed seeds. This combination of characteristics could account for the observed pattern of widely-dispersed, small, isolated populations. Lastly, slow population growth rates appear to occur in related species such as *C. parviflorum* var. *pubescens*. In this taxon, estimates indicate that a seed can require up to 16 years of growth before flowering (Curtis 1954). In other *Cypripedium* taxa such as *C. acaule* Aiton, pollinator limitation may be an important factor in causing low levels of seed set (Davis 1986; Helenurm & Barret 1987; Gill 1989). Pollinator limitation may also play a role at the Virginia site. Out of 120 individuals examined in 1996, no capsules were present from the previous year and floral visitation during 1996 was very low (Case & DeWitt pers. obs.).

Although a number of *C. kentuckiense* sites have been found since 1985, it is our opinion that this taxon should remain a potential candidate for the

federal endangered and threatened species list (i.e., category 2 or, with more documentation of threats to existing populations, elevation to category 1). Only 156 populations are known with the majority (68%) of the populations occurring in only two states, Arkansas and Kentucky. In some states, such as Louisiana, this species seems to be particularly at risk. Approximately 10 Louisiana populations (not included in this study) have been extirpated primarily due to logging and development (Julia Lark pers. comm.). The remaining states listed in Table 1 each contain less than 6% of all populations each. The infrequent and scattered occurrences of *C. kentuckiense* in states ranging from Virginia to Texas may be the remnants of more widely distributed and abundant ancestral populations. In this case, it will be particularly important to categorize the demographic, genetic, and anthropogenic threats to the extant populations, especially in light of the large number of small, apparently isolated populations. Potential future discoveries of populations in new regions, such as the mountains bordering Virginia and West Virginia might be expected. These discoveries could provide important new insights into the distribution and genetic structure of this rare orchid.

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